Research project related to XXXVIII cycle

MOLECULAR AND CELLULAR NETWORK UNDERLYING NEUROGENIC MUSCLE ATROPHY

Skeletal muscle has an extraordinary regenerative capacity thanks to the presence of adult muscle stem cells named satellite cells. Despite the ability of the satellite cells to regenerate the muscle after damage, skeletal muscle regeneration is functionally successful only when the newly formed myofibres become innervated through the establishment of new Neuro-Muscular Junctions (NMJs). The mechanisms that regulate the growth or extension of neurites toward muscle fibers and the timing of functional synaptic reestablishment after damage remain unclear and unexplored. Moreover, loss of NMJ in the intact muscle tissue leads to muscle atrophy consistent with the vital role of NMJ in muscle maintenance and homeostasis. Neurogenic muscle atrophy is a hallmark of several degenerative processes involved in different pathologies, such as Amyotrophic Lateral Sclerosis (ALS). This condition is characterized by progressive motor neurons (MNs) degeneration, which leads to a deterioration of neuromuscular functions, causing weakness, paralysis, and atrophy of skeletal muscles. During neurogenic muscle atrophy, the interruption of transmission of neurogenic signals to muscles, caused by loss of neuromuscular junction (NMJ) integrity, activates protein breakdown and reduces protein synthesis, leading to the loss of muscle mass and contractile activity. Recently we demonstrated that not only muscle fibers are sensitive to muscle innervation. Indeed, disruption of the neuromuscular integrity – due to either traumatic or genetic disease - activate a specific gene expression program in muscle resident cell populations. To analyse the role of these populations in maintaining the cross talk between muscle and nerve, we will use next-generation sequences, in vitro and in vivo studies. With these complementary approaches, we will identify new cellular and molecular players underlying neurogenic muscle atrophy. MAIN METHODS USED: Mouse models of neuromuscular diseases, primary cultures, classical molecular analyses (qPCR, WB), morphological analyses, next-generation sequencing (RNA-seq, scRNAseq, spatial transcriptomics).

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Key publications:

1. Biferali, B., Proietti, D., Mozzetta, C., and Madaro, L. (2019). Fibro-Adipogenic Progenitors Cross-Talk in Skeletal Muscle: The Social Network. Front Physiol 10. https://doi.org/10.3389/FPHYS.2019.01074.
2. Madaro, L., Passafaro, M., Sala, D., Etxaniz, U., Lugarini, F., Proietti, D., Alfonsi, M.V., Nicoletti, C., Gatto, S., de Bardi, M., et al. (2018). Denervation-activated STAT3-IL-6 signalling in fibro-adipogenic progenitors promotes myofibres atrophy and fibrosis. Nat Cell Biol 20, 917–927. https://doi.org/10.1038/s41556-018-0151-y.
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CUSTOM-ENGINEERED EXTRACELLULAR VESICLES TO COUNTERACT MUSCLE DEGENERATIONS

Growth and regeneration under physiological and pathological circumstances are innate characteristics of skeletal muscle. The balanced interplay between anabolic and catabolic processes ensures the overall plasticity of muscle mass, which may be severely disrupted in cases of chronic muscle illnesses caused by genetic and acquired factors. Paracrine factors, such as extracellular vesicles (EVs), secreted by myogenic stem cells have been implied in contributing immensely to the regenerative machinery of skeletal muscles. EVs have been studied as hosts for factors such as proteins and non-coding RNAs, including microRNAs (miRNAs). We identified miRNAs that can boost myogenic differentiation of mesodermal progenitors derived from human

induced pluripotent stem (hiPS) cells and among them miR 181a/miR212 improves myogenic commitment in fusion-negative rhabdomyosarcoma. Specific molecular signatures can be detected in circulating EV cargos that may impact skeletal muscle homeostasis. Preliminary studies showed that EVs originating from hypertrophic mouse models enhance the myogenic potential of muscle stem cells both *in vitro* and *in vivo.* In this doctoral program we wish to elaborate more on these findings and pursue the goal of establishing custom engineered Evs and test them on 2D and 3D cell models based on healthy and Duchenne muscular dystrophy hiPS cells to mimic dystrophic and atrophic muscles. We will establish a DROSHA knockout immortalized human myoblast cell line and characterize their EVs. We will transfect the EVs with several combinations of mimics and antagomirs of relevant miRNAs and focus on the implementation of *in vivo* regeneration through a cell-free method.
Forthcoming therapeutic triumphs, by either delivering EVs with custom-engineered cargos alone, or in combination with stem cell therapy, may represent the future of muscle wasting therapeutics.
APPLIED TECHNIQUES: Ultracentrifugation, cell culture, RT-qPCR, western blot, immunofluorescence staining, transfection, mouse handling.
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Key publications

1. Giacomazzi G, Holvoet B, Trenson S, Caluwé E, Kravic B, Grosemans H, Cortés-Calabuig Á, Deroose CM, Huylebroeck D, Hashemolhosseini S, Janssens S, McNally E, Quattrocelli M, Sampaolesi M. MicroRNAs promote skeletal muscle differentiation of mesodermal iPSC-derived progenitors. Nat Commun. 2017; 8(1): 1249.
2. Pozzo E, Giarratana N, Sassi G, Elmastas M, Killian T, Wang CC, Marini V, Ronzoni F, Yustein J, Uyttebroeck A, Sampaolesi M. Upregulation of miR181a/miR212 Improves Myogenic Commitment in Murine Fusion-Negative Rhabdomyosarcoma. Front Physiol. 2021;12: 701354
3. Natacha Breuls, Nefele Giarratana, Laura Yedigaryan and Maurilio Sampaolesi, “Epigenetic Modifications in Induced Pluripotent Stem Cells to Boost Myogenic Commitment”, in Advances in Stem Cell Biology, volume Induced Pluripotent Stem Cells - Novel Concepts, Editor: Alexander Birbrair, Elsevier, 2022
4. Duelen R, Costamagna D, Gilbert G, De Waele L, Goemans N, Desloovere K, Verfaillie CM, Sipido KR, Buyse GM, Sampaolesi M. Human iPSC model reveals a central role for NOX4 and oxidative stress in Duchenne cardiomyopathy. Stem Cell Reports. 2022; 17(2):352-368.

ROLE OF EPIGENETIC AND METABOLIC REPROGRAMMING IN NORMAL AND NEOPLASTIC HEMATOPOIESIS.

The activity of the group is focused on the mechanistic understanding of the epigenetic programs affecting the DNA, RNA and chromatin status in human hematopoietic cells, whose defects can contribute to the pathogenesis of different myeloid malignancies. The general aim of the proposed project is to link the regulation of the epigenome by nuclear miRNAs and/or m6A RNA modifications to the metabolic reprogramming occurring during normal and neoplastic myelopoiesis. The results of these studies may improve the comprehension of the epigenetic mechanisms regulating myeloid differentiation and can provide promising candidates for the diagnosis, prognosis and for the design of novel therapeutical interventions in specific myeloid malignancies.
Contact-Tutor riferimento: clara.nervi@uniroma1.it
Key publications

1. Quattrocchi A, et al. Genetic lesions disrupting calreticulin-untranslated region in JAK2 mutation-negative polycythemia vera. Am J Hematol. 2020 Jun 22. doi: 0.1002/ajh.25911. PMID: 32572989.
2. Banella C. et al. Ascorbate Plus Buformin in AML: A Metabolic Targeted Treatment. Cancers (Basel). 2022 May 23;14(10):2565. doi: 10.3390/cancers14102565. PMID: 35626170

DYNAMIC AND REVERSIBLE N6-METHYLADENOSINE (m^6A) MODIFICATION DURING MYELOID LEUKEMIA PROGENITOR CELL DIFFERENTIATION

RNA chemical modifications in coding and non-coding RNAs are known from decades. They are generally installed by specific enzymes and, in some cases, they can be read and erased by other specific proteins. The majority of them occurs in transfer RNA (tRNA) and ribosomal RNA (rRNA) while a minority of them occurs in messenger RNAs (mRNA) and long non-coding RNAs (lncRNAs). In all cases RNA modifications may play important role in RNA folding, stability and function. The impact of RNA chemical modifications on gene expression regulation and the reversible nature of some of these modifications led to the birth of the word epitranscriptomics, in analogy with the changes that occur on DNA and histones. Among more than 100 different modifications identified so far, most of the epitranscriptomics studies focused on the N6-methyladenosine (m6A), which is the more abundant internal modification in protein coding RNAs. controlling several pathways of gene expression at the basis of the cell fate determination. Even if most of the m^6A studies focused on its direct role on mRNA function, recent evidences showed that m^6A can also regulate the synthesis and function of ncRNAs and, on the contrary, ncRNAs can also influence the function of m^6A modification in mRNAs, highlighting a novel interplay between m6A and non-coding RNAs functional activity. The functional contribution of m^6A modification on normal and pathological myeloid differentiation will be addressed during the PhD project highlighting the relevance of specific factors, as METTL3 and FTO, for coding and non-coding RNA functions at the basis of myeloid differentiation by using gene silencing technology, chemical inhibitors and novel gene-transfer technology for cell delivery. The PhD student will work in a dynamic and interdisciplinary environment with the possibility of scientific growth.

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Key publications

1. Ianniello Z, et al. New insight into the catalytic -dependent and -independent roles of METTL3 in sustaining aberrant translation in chronic myeloid leukemia. Cell Death Dis. 2021 Sep 24;12(10):870.
2. Palombarini F, et al. Self-assembling ferritin-dendrimer nanoparticles for targeted delivery of nucleic acids to myeloid leukemia cells. J Nanobiotechnology. 2021 Jun 9;19(1):172.
3. Fazi F, Fatica A. Interplay Between N 6-Methyladenosine (m6A) and Non-coding RNAs in Cell Development and Cancer. Front Cell Dev Biol. 2019 Jun 28;7:116.

THE ROLE OF GENDER IN SHAPING THE IMMUNE RESPONSE IN CANCER CACHEXIA AND ITS EFFECT ON THE SKELETAL MUSCLE MICROENVIRONMENT

Cancer cachexia is characterised by progressive weight loss and skeletal muscle atrophy and is associated with increased mortality among cancer patients. In spite of some promising clinical trials, cachexia remains an uncurable disease state. Understanding how cancer alters the complex microenvironment in skeletal muscle is crucial for the development of novel approaches to the treatment of cachexia. Likewise, tailoring cachexia diagnosis and therapy based on the sex of the patient may further increase the efficacy of the therapeutical interventions. The skeletal muscle microenvironment comprises a mixture of mononucleated satellite cells, fibroblasts, endothelial cells, and immune cells. Together, these cells cooperate to maintain muscle homeostasis, mediate repair following injury, and establish systemic communication and cross-talk through the secretion of soluble mediators such as myokines. It is therefore important to gain insight into the mechanism by which multiple cell types interact to alter the muscle's microenvironment during the onset and progression of cancer cachexia, thus contributing to loss of muscle mass and function. Another critical issue that has remained underexplored is the role of gender in determining the development and severity of cachexia. While it is well established that gender has a significant effect on the immune response, less is known about its role in the context of cachexia. Although sex differences have been postulated to occur in cachexia, there is very little evidence demonstrating a dimorphism of the disease state linked to gender. This PhD project will examine in detail the gender specific

differences in the immune response in skeletal muscle in vitro and in vivo using a mouse model of cachexia. Specific questions that will be addressed include how gender influences: (1) the skeletal muscle microenvironment at different stages of cachexia, (2) the source, composition, and kinetics of the immune cells in skeletal muscle, (3) the phenotype and function of the immune cells within skeletal muscle during cachexia onset and progression, (4) the cross-talk between skeletal muscle and primary and secondary lymphoid organs such as the bone marrow and spleen, and (5) the inflammatory mediators released from skeletal muscle resident cells, circulating immune cells or the tumour.

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Key publications

1. Rizzo G, Di Maggio R, Benedetti A, Morroni J, Bouche M, and Lozanoska-Ochser B (2020). Splenic Ly6C^{hi} monocytes are critical players in dystrophic muscle injury and repair. *JCI Insight*, 5: e130807.
2. Berardi E, Madaro L, Lozanoska-Ochser B, Adamo S, Thorrez L, Bouche M, Coletti D (2021). A Pound of Flesh: What Cachexia Is and What It Is Not. *Diagnostics*, 12;11(1):116. doi: 10.3390/diagnostics11010116.
3. Renzini A, Riera CS, Minic I, D'Ercole C, Lozanoska-Ochser B, Cedola A, Gigli G, Moresi V, Madaro L (2021). Metabolic remodelling in skeletal muscle atrophy as a therapeutic target. *Metabolites*, 5;11(8):517.

DEVELOPMENT OF EFFICIENT GENE DELIVERY SYSTEMS FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is an X linked recessive disorder that affects approximately 1 in 5000 live male births. The disease is due to an absence of the dystrophin protein that is critical to the stability and function of myofibres in skeletal and cardiac muscle. Dystrophin gene therapy could be an effective tool for DMD treatment, but it is limited by the size of the gene encoding dystrophin that is the largest known gene in humans (~ 2,4 millions of base-pairs). To overcome this limitation, truncated forms of dystrophin, referred to as mini- and micro-dystrophins, have been developed. Gene therapy using mini- and micro-dystrophin genes induces the production of shorter, but functional, versions of the dystrophin protein and reduces muscle damage in DMD) patients. The delivery of mini- and micro-dystrophin genes can be significantly enhanced by their encapsulation in engineered nanoparticles (NPs). The main aim of the Ph.D. program is the development of novel strategies based on liposomes, lipid NPs (LNPs) and other lipid-based systems for the efficient delivery of mini- and micro-dystrophin constructs and other nucleic acids (*e.g.*, anti-sense oligonucleotides, miRNA). The program is aligned to one of the five technological issues entitled “Development of gene therapy and drugs with RNA technology” launched by the Minister for University and Research under the Italy’s recovery and resilience plan (PNRR). Gene delivery systems will be developed using bulk mixing processes and microfluidic platforms under controlled experimental conditions. Formulations with optimized physical-chemical properties will be screened for transfection efficiency and cell viability *in vitro* using commercial and primary myoblast cell lines. Lead candidates will be validated using an established three-dimensional vascularized *ex-vivo* muscle engineered tissue (X-MET) and, subsequently, mice models of DMD. The selected candidate will work in an interdisciplinary environment under the supervision of biologists, biotechnologists, and physicists.

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Key publications

1. Francesca Giulimondi, Luca Digiacomo, Daniela Pozzi, Sara Palchetti, Elisabetta Vulpis, Anna Laura Capriotti, Riccardo Zenezini Chiozzi, Aldo Laganà, Heinz Amenitsch, MortezaMahmoudi, Isabella Screpanti, Alessandra Zingoni, Giulio Caracciolo. Opsonin-deficient nucleoproteic corona endows unPEGylated liposomes with stealth properties in vivo. ACS Nano, 2022, 16, 2, 2088–2100, doi: 10.1021/acsnano.1

MODULATION OF AROMATASE EXPRESSION IN BREAST CANCER CELLS UPON STIMULATION WITH DCI

The research program aims at investigating the anti-aromatase activity of D-Chiro-Inositol (DCI), an isomer of inositol. Some preliminary data have suggested that DCI can downregulate aromatase activity in ovary cells. We can therefore hipothesize that DCI can also exert an anti-estrogenic activity in different settings, namely endometriosis and breast cancer responsive to endocrine stimulation. However, this effect should be examined in a proper 3D-context, given that collagen concentrations, as well as the partecipation of other microenvironment components, can significantly interfere with the endocrine modulation. The program shall investigate in a 3D-model of normal/cancerous breast cancer cells, how endocrine pathways (namely the peripheral activation of aromatase and the subsequent increase in estrogens) could be efficiently modulated by different DCI concentrations. The study will investigate the synthesis and the post-traslational activation of aromatase (by reconstructing changes in the genome expression partner), the consequent delivery of sex hormones (androgens, estradiol and estrone), and the associated modifications of main pathways involved in cell proliferation, motility and invasiveness. These effects will be correlated with the features of the different microenvironments on which the 3D-model shall be built in order to assess how the pharmacological activity of inositol could be blunted/enhanced by context-dependent factors.

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Key publications

1. Jalouli M, Mofti A, Elnakady YA, Nahdi S, Feriani A, Alrezaki A, Sebei K, Bizzarri M, Alwasel S, Harrath AH. Allethrin Promotes Apoptosis and Autophagy Associated with the Oxidative Stress-Related PI3K/AKT/mTOR Signaling Pathway in Developing Rat Ovaries. Int J Mol Sci. 2022 Jun 7;23(12):6397. doi: 10.3390/ijms23126397.
2. Laganà AS, Forte G, Bizzarri M, Kamenov ZA, Bianco B, Kaya C, Gitas G, Alkatout I, Terzic M, Unfer V. Inositols in the ovaries: activities and potential therapeutic applications. Expert Opin Drug Metab Toxicol. 2022 Feb;18(2):123-133. doi: 10.1080/17425255.2022.2071259.

EXPLOITING TPC2 ENDOLYSOSOMAL/MELANOSOMAL CHANNEL TO OVERCOME MALIGNANT MELANOMA DRUG RESISTANCE: THERANOSTICS THROUGH BRIDGING OF NON INVASIVE CUTANEOUS IMAGING AND MOLECULAR PROFILES OF TUMOR CELLS

Malignant melanoma (MM), a highly aggressive skin cancer, is a complex and heterogeneous disease presenting genetic, clinical, and histopathological variations responsible for unpredictable treatment efficacy. BRAFV600E is the most common oncogenic mutation but the beneficial effects of BRAF inhibitors on patient survival are arrested by acquired resistance. Recently, by using Reflectance Confocal Microscopy (RCM), our collaborators from University of Modena and Reggio Emilia reported a close correlation between the characteristics of the four primary melanoma subtypes identified by this innovative imaging approach and specific genetic signatures linked to tumor aggressiveness. It has been observed that melanosomes released by melanoma cells promote the formation of a dermal tumor primary niche that facilitates metastatic progression. Two-Pore Channel 2 (TPC2) is an ion (Na+/Ca2+) channel localized on the membrane of intracellular acidic compartments, including melanosomes, playing a role in the aggressiveness of different cancer types. Notably, we reported that in patients' dataset analysis as well as in MM cell lines, expression of this channel in primary vs. metastatic phenotype is different and plays different roles. This apparent dual role in MM asks for the identification of the underlying mechanisms and TPC2 metastatic potential. Our working hypothesis is that screening different phenotypes of MM by RCM for the expression and activity of this channel will be highly informative both for patients' stratification and for disclosing a novel possible therapeutic target, to be inhibited or activated depending on the genetic background, microenvironment, and the stage of the disease progression. The impact of TPC2 on tumor cells and microenvironment will be studied in MM

cell lines and in primary cultures derived from biopsies of MM patients, whose subtypes have been defined by RCM imaging technology and characterized by spatial transcriptomics.
These studies will reveal a robust network of information where TPC2 expression will be related to the features of MM subtypes and their metastatic potential and will provide a basis for developing molecular targeted therapies. The most important aim of this study is to contribute to the development of theranostics, the new strategies that combine non-invasive imaging-based diagnostics with therapy to bypass tumour drug resistance.
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Key publications

1. D'Amore A, Hanbashi AA, Di Agostino S, Palombi F, Sacconi A, Voruganti A, Taggi M, Canipari R, Blandino G, Parrington J, Filippini A. Loss of Two-Pore Channel 2 (TPC2) Expression Increases the Metastatic Traits of Melanoma Cells by a Mechanism Involving the Hippo Signalling Pathway and Store-Operated Calcium Entry. Cancers (Basel). 2020 Aug 24;12(9):2391. doi: 10.3390/cancers12092391
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TARGETING BCL-2 PROTEIN IN COMBINATION WITH TARGET THERAPY AND IMMUNOTHERAPY TO IMPROVE MELANOMA TREATMENT

Metastatic melanoma is one of the most highly mutated, molecular heterogeneous and lethal type of cancer. The most prominent genetic alterations driving melanomagenesis result in the constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, with BRAF and NRAS hot-spot mutations accounting for about 50% and 30% of all melanoma cases, respectively. To date, the standard-of-care for BRAF mutant metastatic melanoma includes the BRAF and MEK inhibitors (BRAFi/MEKi) combination therapy or immunotherapy with anti-PD-1, anti-CTLA-4, or the combination of the two immune checkpoint inhibitors (ICI). Unlikely, therapeutic options are still limited for patients without BRAF mutations and most patients treated with BRAFi/MEKi develop resistance by multiple mechanisms that, in the majority of cases, results in the re-activation of MAPK pathway or in the up-regulation of other pro-survival signaling pathways. Therefore, the development of new therapeutic combinations is an urgent need to improve the outcome of patients. In this context, the bcl-2 oncogenic network is one of the most crucial regulators of melanoma cell apoptosis involved in therapeutic resistance. The objectives proposed in this project could put the rationale for the use of bcl-2 as a therapeutic target in melanoma. Supported by our published data and preliminary results, the research described in this proposal is addressing a high-unmet medical need and is expected to generate the following outputs with both short- and long-term impacts on the clinical management of advanced melanoma: i) advance in understanding bcl-2 cellular functions, with particular regards to its ability to promote PD-L1 expression, ii) identification of proof-of-concept data in support of new combination therapies to be translated to clinical application for melanoma, and iii) identification of new robust biomarkers that may predict therapy response. Clinical translational impact of the project will be achieved by using a clinically annotated BioBank of melanoma specimens, to correlate the expression of bcl-2 and patient response to ICI or target therapy. In addition, considering that a fraction of patients with metastatic melanoma has a reduced life expectancy or no further treatment options after developing resistance to therapy, the development of new drug combinations, that can delay or overcome this resistance, could open new therapeutic opportunities for these patients.
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Key publications

1. Di Martile M et al. Melanoma-specific bcl-2 promotes a protumoral M2-like phenotype by tumor-associated macrophages. J Immunother Cancer. 2020

2. Valentini E et al. Targeting the anti-apoptotic Bcl-2 family proteins: machine learning virtual screening and biological evaluation of new small molecules. Theranostic, 2022 DOI:10.7150/thno.64233.

BIOMIMETIC 3D PRINTED SCAFFOLDS FOR THE TREATMENT OF THE SKELETAL SYSTEM PATHOLOGIES.

The musculoskeletal system represents the scaffolding for the mammalian body and the bones provide attachment sites for muscles, tendons, and ligaments, enabling locomotion, as well as being critical to adult hematopoiesis activity. In recent years, due to the ageing of the population, the incidence of bone diseases is increasing; thus osteoporosis, arthritis, cancer etc. are becoming the first cause of disability. Some consequences of these diseases are bone fragility, which leads to a high risk of fracture, and critical bone defects with clinically difficult within orthopedics. These degenerative pathologies when have a critical size of about 1-2 cm and loss of more than 50% of the circumference, can compromise the intrinsic bone regenerative capacity and it is necessary to intervene with standard clinical protocols. The current method used are autograft (golden standard), allograft and xenograft; each of these techniques has advantages but also many disadvantages such as infections, high morbidity rate at the donor site (autograft and allograft), disease transmission and rejection (allograft and xenograft) and cost for all treatments. In this scenario, tissue engineering can pave the way to an innovative approach to overcome the limitations collected so far in treating bone pathologies by the development of specific and smart scaffolds. Bone tissue engineering strives to replicate the osteoconductive, osteoinductive and osteogenic properties of bone as well as ensuring mechanical strength and biocompatibility. Three-dimensional biodegradable structures able to stimulate the healthy tissue regeneration during the degradation phase, avoiding the release toxic by-products to the host organism, can be thus regarded as a valuable option to be critically assessed. In addition, 3D printing for tissue engineering applications offers the possibility to design scaffolds characterized by a fine, porous and disordered microarchitecture mimicking the morphology of the natural extracellular matrix.

The project aims to design, through this biomimetic approach, ad hoc scaffolds for bone tissue engineering and evaluate their biocompatibility, osteoconductive and osteoinductive properties through the study of cell-material interaction dynamics. Bone ECM-like scaffolds will be fabricated by fused deposition modeling (FDM) and tested by means of both osteoblastic-like cells and mesenchymal stem cells. In particular, the biological response will be investigated by analysis of cell growth, metabolism, morphology, and differentiation capability of both cells models seeded on different types of 3D printed polylactic acid (PLA) scaffolds (mimicking physiological and pathological conditions). Moreover, the interaction between cells and the scaffolds will be studied in standard static conditions and dynamic ones, using a microgravity Rotary Cell Culture System, to reproduce in vitro 3D growth as close as possible to in vivo physiological and pathological conditions. The aim of the study will be to obtain functionally compatible platforms for regenerative medicine applications of bone damage, capable of replicating the three-dimensional structure of the bone and promoting its regeneration.

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Key publications

1. Ledda M. et al. Biological Response to Bioinspired Microporous 3D-Printed Scaffolds for Bone Tissue Engineering. Int. J. Mol. Sci. 2022, 23, 5383. https://doi.org/10.3390/ijms23105383.
2. Mussi V. et al. Raman Mapping of Biological Systems Interacting with a Disordered Nanostructured Surface: A Simple and Powerful Approach to the Label-Free Analysis of Single DNA Bases. Materials Science and Engineering: C, 2021; https://doi.org/10.1016/j.msec.2021.111951.
3. Ledda M. et al. Biocompatibility assessment of sub-5 nm silica-coated superparamagnetic iron oxide nanoparticles in human stem cells and in mice for potential application in nanomedicine. Nanoscale, 2020; DOI: 10.1039/c9nr09683c

4. Ledda M et al. Interdisciplinary approach to cell-biomaterial interactions: biocompatibility and cell friendly characteristics of RKKP glass-ceramic coatings on titanium. Biomed. Mater. 10 (2015) 035005

ROLE OF THE DIFFERENT INTRACELLULAR PATHWAYS IN THE STUDY OF THE BIOGENESIS OF EXOSOMES

Exosomes are a subtype of small extracellular vesicles (EV) secreted by most eukaryotic cells, that play a fundamental role in intercellular communication. They can be distinguished from other types of EVs in that they originate in the late endosomal compartment and carry specific cargoes capable of influencing both physiological and pathological processes in recipient cells. Exosomes are formed within late endocytic compartments or multivesicular bodies (MVB) by invagination of the limiting membrane into the lumen. Intraluminal vesicles (ILV) accumulated in MVB are then released in the extracellular space by fusion with the plasma membrane. The process of exosome biogenesis is the result of several mechanisms that involve the endosomal sorting complex required for transport (ESCRT). ESCRT is a multimolecular machinery composed of several proteins that facilitate the curvature of the membrane and the formation of vesicles. Recent evidence favors an alternative pathway for sorting exosomal cargo into MVBs, which seems to depend on raft-based microdomains characterized by high levels of cholesterol and sphingolipids for the lateral segregation of cargo within the endosomal membrane. All these processes regulate the sorting in the exosomes of biologically active molecules such as proteins, lipids and nucleic acids (mRNAs, miRNAs and non-coding RNAs). The composition of the exosome is highly heterogeneous depending on the cellular origin and pathophysiological status, which suggests that the recruitment of contents into the exosome is a highly regulated process. The aim of this project is to dissect the mechanisms of exosome biogenesis using an innovative methodology to obtain metabolically labeled fluorescent exosomes that can be accurately traced and counted. The use of a series of inhibitors of vesicular transport and of different pathways involved in the formation of membranes along with the analysis of the lipidomic and proteomic profiles of the secreted exosomes will allow to expand the knowledge on the mechanisms of exosome formation whose understanding is of particular importance in the study of tumor progression and in the regulation of the immune response.

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Key publications

2. Fiani, M. L., V. Barreca, M. Sargiacomo, F. Ferrantelli, F. Manfredi and M. Federico (2020). "Exploiting Manipulated Small Extracellular Vesicles to Subvert Immunosuppression at the Tumor Microenvironment through Mannose Receptor/CD206 Targeting." Int J Mol Sci 21(17).
3. Raggi, C.; Diociaiuti, M.; Caracciolo, G.; Fratini, F.; Fantozzi, L.; Piccaro, G.; Fecchi, K.; Pizzi, E.; Marano, G.; Ciaffoni, F.; Bravo, E.; Fiani, M. L.; Sargiacomo, M., Caveolin-1 Endows Order in Cholesterol-Rich Detergent Resistant Membranes. Biomolecules 2019, 9 (7).
4. Zanetti, C.; Gallina, A.; Fabbri, A.; Parisi, S.; Palermo, A.; Fecchi, K.; Boussadia, Z.; Carollo, M.; Falchi, M.; Pasquini, L.; Fiani, M. L.; Sargiacomo, M., Cell Propagation of Cholera Toxin CTA ADP Ribosylating Factor by Exosome Mediated Transfer. International journal of molecular sciences 2018, 19 (5), 1521.
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LE PERSONE INTERESSATE ALLE LINEE DI RICERCA SOPRA RIPORTATE E CHE VOLESSERO AVERE MAGGIORI DETTAGLI POSSONO CONTATTARE DIRETTAMENTE I RESPONSABILI SCIENTIFICI (TUTOR DI RIFERIMENTO)